REMARKS / ARGUMENTS

Support for Amendments

Claims 1 and 72 are amended to recite the gap is at least 3 microns wide. Support for the amendments may be found throughout the specification and drawings as originally filed including paragraph [00173], which recites in part,

> "Preferably, the width of a gap between electrodes or electrode elements of a device of the present invention used for monitoring eukaryotic cells, such as mammalian cells, such as cancer cells, endothelial or epithelial cells, is between about 3 microns and 80 microns, more preferably between about 5 microns and 50 microns, and most preferably between about 8 microns and 30 microns."

Response to Claim Rejections Under 35 U.S.C. § 103

I.

Claims 1-3, 7-13, 15, 25, 26, 36, 38-40, 43, 72, 287, 288, 290-297, 309 and 310 Are Not Obvious Over Wolf (US 6280586) in View of Gerwen (US 6440662)

The examiner has rejected claims 1-3, 7-13, 15, 25, 26, 36, 38-40, 43, 72, 287, 288, 290-297, 309 and 310 under 356 U.S.C. §103(a) as allegedly being obvious over Wolf in view of Gerwen. Specifically, the Examiner states that with respect to claims 1, 8, 9, 36, 43, 72, 287 and 288, 290-293, 309 and 310, Wolf discloses a device for detecting cells comprising a non-conductive substrate (Figure 2:5) having two opposing ends, and a plurality of electrode arrays positioned on the substrate. Each electrode array comprises at least two electrodes (Figure 2:10), and electrically conductive traces and connection pads are in communication with the electrode arrays. The electrodes are used to detect impedance changes resulting from attachment of cells to the electrode surface. This is described in column 2, lines 39-55, column 3, lines 11-28, and column 7, lines 29-50. Column 7, line 63 to column 8, line 12 indicates that the electrodes have a surface (Figure 5:13) that is suitable for cell attachment and growth. Wolf, however does not

expressly state that the electrodes in each array have a width of more than 1.5 to 15 times the width of the gap between the electrodes.

The Examiner then newly cites Gerwen in support of an electrode having a width from 1.5 to 15 times the width of the gap. Specifically, the Examiner argues Gerwen discloses an impedimetric detection system that comprises a plurality of interdigitated electrodes. Detection of an analyte is determined based on the interference of an electrical field between the electrodes. This is disclosed in column 3, line 40 to column 4, line 11 and in column 10, lines 40-52. The Examiner further refers to Figure 1C as showing that the width of the electrodes is between 1.5 to 15 times the width of the gap between electrodes.

The Examiner alleges Wolf and Gerwen are analogous art because they are from the same field of endeavor regarding microelectronic cell sensor devices.

The Examiner concludes that it would have been obvious to one of ordinary skill in the art at the time the invention was made to alter Wolf's device to ensure that the electrode widths were more than 1.5 and less than 15 times the non-conductive material width if it was determined through trial and error that this configuration produced the best results. This limitation is considered to be a result of effective variable that is optimized through routine experimentation. This position is supported by Gerwen, who indicates that electrode width and gap sizes all depend on several considerations that involve engineering tradeoffs. Gerwen states in column 3, lines 1-8 and column 4, lines 1-11 that a small gap to electrode width radius results in a high degree of miniaturization and an increase in sensitivity.

Applicants indicate differences between Wolf, Gerwen and Applicants' invention below to demonstrate the different fields of endeavor. However, to expedite allowance of the present application, Applicants have amended claims 1 and 72 to newly include the gap width is at least 3 microns wide. Neither Gerwen or Wolf alone or in combination provide this element.

A. Gerwen is Not Analogous Art to Wolf or to the Claimed Invention Because the Inventions Are in Different Fields of Endeavor

While both Gerwen and Wolf broadly include impedimetric devices, Gerwen is directed towards devices for detecting molecular binding events; whereas Wolf is directed towards devices for detecting the presence of biological or chemical components in an analyte through the measurement of cells. For completeness, Applicants' invention is directed towards devices and methods for detecting or monitoring cell attachment or growth.

Systems for detecting molecular binding events and systems for detecting whole cells seek to answer different scientific questions, involve significantly different technical hurdles, and have thus developed field specific designs, solutions and applications. Accordingly, devices for detecting molecular binding events and those directed towards cell-based applications are considered different endeavors by those skilled in the present art. Moreover, Gerwen seeks to develop a system specifically addressing deficiencies in the detection of molecular binding events. Thus it would not be proper to combine Gerwen with a cell-based system.

A1. Gerwen attempts to address particular scientific hurdles inherent in the detection of binding interactions between similarly sized molecules; whereas the significant difference in size between cells and molecules do not result in such challenges in cell-based systems

Gerwen is directed towards methods of detecting binding reactions between molecules. Specifically, referring to column 1, lines 6-10 of Gerwen,

> "The present invention relates to an improved sensor for electronically detecting a binding reaction between molecular structures or a pair of chemical substances, such as oligonucleotides, antigens, enzymes, peptides, antibodies, DNA and RNA fragments."

A primary object of Gerwen is to reduce background noise generated from unbound molecules free in solution; however such a condition is not found in cell-based systems. In Gerwen, since both bound and unbound molecules are similar in size both will affect the electric field similarly. Thus the presence of unbound molecules within

the electric field generates additional signal as background noise. Gerwen specifically addresses this as a problem requiring a solution in column 2, lines 61-67,

> "This is a major drawback especially when the region of interest is limited in space, i.e. it is an enzymatic or polymeric membrane or an adsorbed molecular layer at the surface of the structure. Any field line depassing this region of interest, introduces in the impedimetric response a shunting impedance which can be considered as noise for the measurement."

Gerwen attempts to solve the problem by attempting to lower the height of the electric field. By lowering the height of the field, Gerwen believes the detectable population of unbound molecules is reduced. The result is believed to be a decrease in background noise. Gerwen proceeds to indicate for molecular detection systems a planar substrate is limited in its ability to lower the electric field. Therefore according to Gerwen, planar systems are not desirable for molecular binding systems and Gerwen abandons such a design. Referring to column 2, lines 55-61,

> "Another important characteristic of the microelectronic technology is its planarity: the microelectrodes patterned this way are essentially flat elements. This feature is not a strong point in the impedimetric devices. In a planar impedimetric structure the electric field lines expand more above the device surface and out of the region of interest in comparison to real 3-D structures."

In contrast, the height of the electric field is not a problem in cell-based systems. In cell-based systems, unbound molecules do not provide measurable variations in impedance. Cells are many times greater in size than molecules and thus contribute significantly more to impedance measurements. In other words because cells are much larger than molecules there is no measurable background noise due to the presence of molecules free in solution. Since such a problem is not found in cell-based systems, the Gerwen reference would be considered in a different field of endeavor and would not be considered by one skilled in the art.

A2. Gerwen attempts to address technical hurdles specific to molecular binding reactions on a nanometer scale, which are not found in cell-based systems

Another primary object of Gerwen is to provide detection of binding reactions on a nanometer scale. As indicated above, Gerwen attempts to detect binding between molecules such as DNA fragments, enzymes, antibodies, etc, which are nanometers in size. In other words, Gerwen operates on a submicron level. In contrast, cell-based systems operate on a scale of many microns. Such magnitudes of scale are not simply interchangeable but instead involve different challenges including those related to sensitivities, manufacturing and the like.

In addition, the molecular binding systems operate across three dimensions, resulting in additional difficulties in positioning of molecules for proper recognition and binding. Specifically, binding between molecules can be dependent on a particular conformation or dependent on three-dimensional positioning of binding pairs. In contrast cell membranes are highly fluid providing few requirements for positioning. Thus such three-dimensional positioning challenges are often not found in cell-based systems.

A3. The significant differences in electrode design between molecular binding-based and cell-based detection systems reflect the technical hurdles specific for each and thus demonstrate each is in a different field of endeavor

As discussed above, Gerwen attempts to address problems specifically associated with the detection of molecular binding interactions, which are not required in cell-based systems. Such problems result in a significantly different electrode design. For example, Gerwen addresses technical hurdles corresponding to the detection of molecules on the nanometer scale and the development of such devices. Referring to column 3, lines 16-26,

> "However, the reproducibility is low due to somewhat random behavior of the fabrication process, i.e., the Pt deposition and the immobilization procedure.
>
> A true electrode patterning process is likely to insure a good reproducibility of the structures and to improve the control upon sensor behaviour. Devices with patterned features, said features having dimensions of hundreds of nanometers are expected to be highly sensitive

> to DNA fragments of 300 bases, i.e. Exhibiting a total molecular Tenth of about 180 nm, or to other large molecules like enzymes or antibodies (tens of nanometers diameter)."

Accordingly, Gerwen's first solution is to provide a device with patterned features having nanometer or submicron dimensions. In contrast, cell-based systems do not operate on the nanometer scale. Eukaryotic cells are typically in the order of 10 microns or greater than 10 microns. Therefore the solutions provided in Gerwen, nanometer patterned features, would not be desired for a cell-based assay because cells are much larger . Thus the problem and corresponding solution in Gerwen is specific to the field of endeavor of detection of molecular binding interactions. For clarity, Applicants amend claims 1 and 72 to recite the gap is at least 3 microns wide. Applicants again note Gerwen provides submicron dimensions.

Gerwen's second solution also highlights significant differences between the fields of endeavor. Gerwen develops submicron channels to attempt to lower the height of the electric field. Specifically, Gerwen proposes a submicron channel design with a metal coating along a sidewall of the channel. Specifically referring to column 3, lines 45-54,

> "The present invention relates more particularly to a sensor for identifying molecular structures within a sample solution is disclosed. The sensor comprises an insulating layer with a plurality of interspaced channels therein having essentially the same direction. Said channels have a bottom and at least two opposite side-walls along said direction. The channels furthermore have submicron dimensions. A metal coating is applied on one of said two opposite side-walls of essentially each channel and on top of the insulating layer in between said channels thereby forming an impedimetric device with said sample solution within and between the channels."

The submicron channel configuration in Gerwen and the use of a metal coating along one of the side walls permits detection of submicron molecular binding within the channel and highlights a significant difference in approaches between molecular-based and cell-based systems. Referring to column 10, lines 52-62,

> "Due to the sub-micron dimensions of the channels and due to the shape of the electrodes (emerging from the deposition of metal under an angle), the electric fields (11) strongly penetrate in the region with the immobilized probes (11). An even stronger confinement of the electrical fields in the region of interest

would be achieved in case when a second insulating layer is put on top of the (4) and (5) planes. In this way the electrical field lines probe more the interior of the channels where the bound analyte occupies most of the space."

In contrast, cell-based systems use generally planar surfaces and are not significantly affected by free molecules. Accordingly, cell-based systems do not direct an electric field into a channel for detection. In fact, since cells grow on planar surfaces channels would be deleterious to a cell population. Thus, those skilled in the art would not adapt a channel design for cellular studies or those characteristics used in conjunction with Gerwen's channel design. Therefore, Gerwen address problems specific to the detection of molecular binding and proposes solutions specific to his field of endeavor.

Now turning to Wolf, Wolf provides a device for measuring the presence of a component contained in an analyte. The device includes, among other elements, at least one reference sensor and at least one electrical sensor and a microporous interlayer. Wolf does not address particular electrode gap to width ratios. Referring to column 2, lines 1-19 of Wolf,

> "This object is accomplished in that provided between the receptor cells and/or target cells and the measurement structure is a structured, micropourous interlayer which the target cells and/or receptor cells accept as neighbor for adherence."

In summary, while Gerwen and Wolf have broadly applied impedance-based devices for their particular field of endeavor, each field is significantly different. This can be demonstrated by the difference in scientific areas each explore, the significant difference in problems encountered in studying the desired area and the corresponding solutions, which result in significant differences in electrode design and in application. Thus, Gerwen and Wolf are not analogous art. Moreover these are not analogous to Applicants' invention.

For completeness, Applicant notes claim 1 of the present invention (from which claims 2, 3, 7-13, 15, 25, 26, 36, 38-40, 287, 288, 290-297 depend), is directed towards a device for detecting cells on an electrode surface and not for the detection of binding between molecular structures. Claim 72 (from which claims 309 and 310 depend) is

directed towards a device for monitoring cell-substrate impedance and not to detect binding between molecular structures.

Furthermore, to expedite allowance of the present application, claims 1 and 72 have been amended to recite the gap is at least 3 microns wide. Neither Gerwen or Wolf disclose this limitation whether alone or in combination.

B. <u>One Skilled in the Art Would Not Look to Gerwen to Optimize the Apparatus in Wolf Because Gerwen Addresses Design Flaws Specific to the Detection of Molecular Interactions and Not Cell-Based Systems</u>

On page 13 of the Office Action, the Examiner acknowledges Gerwen is directed towards molecular interactions. Therefore it is acknowledged that Gerwen is provided for a significantly different purpose. Nonetheless the Examiner sets forth a consideration that while provided for different purposes they remain analogous art because one of ordinary skill in the art would look to Gerwen for optimizing the apparatus of Wolf. Specifically, referring to page 13 of the Office Action,

> "Although Gerwen teaches that the apparatus is specifically designed to measure the presence of a specific enzyme and/or nucleic acid, one of ordinary skill in the art would understand that Gerwen's apparatus could be used to monitor cell binding according to the same basic principles. Just as the binding of biochemical compounds affects the conductivity between electrodes so would the binding of a cell. Accordingly Gerwen and Wolf are analogous art, and one of ordinary skill in the art would know to look to the teachings of Gerwen regarding electrode configuration when optimizing the apparatus of Wolf."

Applicants incorporate by reference the above discussion of Gerwen and Wolf. One would not seek to combine Gerwen and Wolf to solve cell-based problems because Gerwen addresses technical problems specific to molecular binding interactions and not cells. Specifically, Gerwen seeks to solve problems associated with submicron or nanometer detection, including background noise associated with similarly sized molecules that are free-floating in the solutions above the electrode surfaces. In summary, cell-based systems do not have these problems since cell-based systems operate on a much larger scale and unbound molecules do not affect cellular measurements. Since the technical hurdles addressed by Gerwen are not present in cell-

based systems one skilled in the art would not combine Gerwen and Wolf. However, to further expedite allowance of the present application, Applicants have amended claims 1 and 72 to recite the gap is at least 3 microns wide.

C. Gerwen Does Not Discuss the Ratio of Electrode Width to Gap Width

Referring to page 4 of the Office Action, the Examiner sets forth the following reasoning in the obviousness rejection,

> "Gerwen states in column 3, lines 1-8 and column 4, lines 1-11 that a small gap to electrode width ratio results in a high degree of miniaturization and an increase in sensitivity."

Column 3, lines 1-8 is provided below:

> "Still, depending on the electrodes geometry, i.e. dimensions and interspacing, a big majority of the total signal is enclosed in a certain region above the surface of the device as shown in FIG. 1. From the same figure one can deduce that miniaturisation, i.e. L decrease, is crucial in obtaining impedimetric planar structures that probe the space in the very close neighbourhood of the device."

Applicants' invention specifically relates the ratio of the width of the gap to the width of the electrode and has determined that the specific width ratios are of great importance. When examined closer and in view of Applicants' previous discussion, the above passage does not relate to specific ratios but instead provides support for Gerwen's argument regarding why one should not utilize a planar substrate for the detection of molecular binding and why one should use a channel system. Again, Gerwen finds planar substrates inadequate for the detection of molecular structures and has therefore developed a channel system. Gerwen sought to reduce the height of the electric field due to noise from free molecules. Such problems or corresponding solutions are not present in cell-based systems.

In the passage, the term "L" refers to the width of the electrodes plus the width of the spacing. Thus it is not the ratio of electrode width to spacing width that is of significant importance but instead the total width "L" (electrode width plus spacing width). In Gerwen, minimizing L is of importance for molecular binding detection

because molecules are very small (requiring a high degree of miniaturization). Gerwen was not able to generate a desired field using the planar substrate because the field would expand "out of the region of interest." Thus Gerwen does not identify that the ratio of electrode width to gap width provides a desirable feature but instead he provides a design of sub-micron, 3-dimensional structures for the sensor device.

Moreover, one skilled in the art would not look to modify a cell-based system by viewing Fig. 1A-1C. In Gerwen, Fig. 1A – 1C, "L" is compared with the field-penetration depth. However, it is not clear under what conditions such electrical field penetration is analyzed. Does this refer to the electrical field penetration with the presence of, or without the presence of the probe molecules on the electrode plane surface? Or does this refer to the case with the presence of probe molecules on the surfaces AND with the binding molecules in the solutions? Furthermore, at the interface between the electrode surface and the solution, there is an important layer of ionic double layer, which plays a critical role in the field penetration for the electrode geometries. It is not clear whether such a double layer was taken into account for the field penetration. In addition, it is not clear the meaning of "70%" labeled on the figure. Assume that 70% means 70% of the maximum field. Then, since the gap is Fig 1C is about 10 times smaller, then the maximum field at the electrode edge of the Fig 1C is much larger than, probably about 10 times of, the field in Fig. 1A. Thus, even at height of 70% of maximum field, the TRUE electrical field in Fig. 1C can actually be larger than that in Figure 1A. Then, one could say that the field in Fig. 1C may penetrate as high as the field in Fig. 1A. Regardless these situations, Gerwen does not identify any of the three Figs to provide desirable features for his impedance measurement. And, very importantly, the electrical field distribution in the applicant's invention with cells attached to the electrode surfaces would be significantly different from the cases of molecule-binding occurring to the electrode surfaces or in the electrode gaps. First, in molecule bindings, the binding molecules may float in 3-D in the solutions above the electrode planes. All these molecules, whether or not they are specific for the probes on the electrode/substrate plane, would interact with the electrical field. This may present problems for the impedance detection and would result in "background noise" for the measurement, as discussed above for Gerwen, if these molecules do not specifically bind

to the probes, because the technology is ONLY interested in the binding of the molecule bindings of interactions at the surface. This is contrast to Applicants' cells attachment to the electrode surfaces. Cells in the media would land and attach to the electrode surfaces. There are no cells floating around which would cause such problems. Secondly, the impedance change in molecule binding is a result of the overall molecules interacting three-dimensionally with the field. For cells attached on the electrodes, insulting cell membranes play critical roles and would interact with and interfere with the double layer at the electrode/solution interface, and the membrane would block the ionic current. The electrical field distribution in such cases is totally different from those of the field distribution for molecule bindings. Thus, on one hand, Gerwen does not identify any electrode-gap ratio provides desirable feature and on the other hand, Gerwen's field penetration analysis would not be applicable to the cases in the present invention where cells with insulating cell membranes would attach to the electrode surfaces.

Now referring to the second passage cited by the Examiner (column 4, lines 1-11),

> "The present invention overcomes the problem of sensitivity compared to prior art sensors and methods. One important feature of this novel design is the high degree of miniaturisation. This is likely to reduce the noise of the structure and subsequently to increase its sensitivity. Another remarkable feature of the proposed sensor is its tridimensional geometry. This improves the electric field penetration in the area of interest with an eventual sensitivity increase. Said sensor has an interdigitated electrode structure which can be fabricated in a cheap way, even for large active areas."

This passage again addresses the insufficiencies in planar impedimetric devices and not gap width to electrode width ratios. Specifically since using planar devices "expand out of the region of interest" Gerwen developed a tridimensional geometry with miniaturized, sub-micron features.

Miniaturization is also addressed in the passage. However, miniaturization does not refer to the ratio of the electrode width to the gap but instead, "L" or the combined width of the electrode plus the gap. This is because of the Gerwen device is to be used on an extremely small scale.

Therefore Gerwen does not discuss the ratio of electrode width to gap width as a solution to a problem but instead proposes a channel design, which significantly differs from Applicants' invention.

D. Examining the Objective Evidence in Gerwen and Applicant's Invention Supports Applicants' Position that the Gap to Electrode Width Ratio does not Result from Routine Experimentation

For clarity that Applicants have developed a cell-based system, Applicants have amended claims 1 and 72 to recite the width of the gap is at least 3 microns wide. The ratios and widths were determined specifically for cellular systems, which significantly differ from molecular binding systems

Applicants' development of the present system included more than routine optimization or the incorporation of known technologies. Many scientific hurdles were encountered and the solutions to which reached far beyond what would be expected by one skilled in art with respect to a determination of obviousness. The technical problem that the instant invention solves is to propose a device

- with improved impedance signal upon cell attachment to an electrode surface, and
- which ensures a somewhat similar impedance signal irrespective of the location where the individual cells are attached on the electrode surface, on the electrode center or the electrode edge.

This was achieved by the Applicant by providing a device according to claim 1 in which the electrode element width is more than about 1.5 and less that about 15 times the width of the gap between electrode elements.

The applicant's invention of the present electrode system included important design considerations and significant developments. Below are some examples of these discussions from the application. Referring to paragraph [00173], extensive consideration was required with respect to cell size and gap between electrode elements,

> "For monitoring the behavior of cells, preferably, the gap between electrode elements does not substantially exceed the size (e.g. width of cells when they spread and attach on the substrate) of cells whose behavior is to be monitored using the device. This reduces the possibility that contact between a cell and a substrate occurs without the cell contacting at least a portion of an electrode or electrode element. Further, the width of the gap between electrode elements (or the gap size) preferably is not substantially less than the size of cells (e.g. width of an average cell when it spreads and attaches to the substrate) whose behavior is to be monitored using the device, to reduce the possibility of a cell contacting two neighboring electrode elements is measured and thereby giving rise to a somewhat disproportionately large impedance signal, in comparison to a cell contacting only one electrode element. This is particularly important, if the electrode width is much larger (e.g. ten times) than the size of cells whose behavior is to be monitored using the device. On the other hand, if the electrode width is in comparable with the size of cells (e.g. width of an average cell when it spreads and attaches to the substrate), the width of the gap between electrode elements can be somewhat smaller than the size of cells. While other gap dimensions may be used, preferably, the gap between electrode elements of the electrode structures ranges from about 0.2 times and 3 times the width of an average cell used in an assay using the device. Preferably, the width of a gap between electrodes or electrode elements of a device of the present invention used for monitoring eukaryotic cells, such as mammalian cells, such as cancer cells, endothelial or epithelial cells, is between about 3 microns and 80 microns, more preferably between about 5 microns and 50 microns, and most preferably between about 8 microns and 30 microns."

In addition to the relationship between cell size and gap width between electrode elements, the inventors of the present invention were required to consider the width of electrode elements themselves. Some of the considerations of width of electrode elements in relationship to the electrode resistance in the array and in relationship to cell size were discussed in paragraph [00174], which recites,

> "The width of an electrode element is preferably not too narrow since the resistance of the electrode elements will increase as the width of the electrode element decreases. The increased resistance along the electrode elements will cause a large electrical potential difference between different points along the electrode element, resulting in difference impedance signals for cells landed on and attach to different regions of the electrode elements. It is preferred that cells landed on and attached to any region on the substrate surfaces give similar impedance signals. Thus, for an electrode element that is part of an interdigitated electrode structure or concentric electrode structure, where the device is to be used for

monitoring eukaryotic cells, such as mammalian cells, such as cancer cells, endothelial or epithelial cells, the electrode width is preferably greater than about 3 microns, and more preferably greater than about 10 microns. The width is also limited by the consideration that if an electrode element is very wide, a cell that is positioned over a central part of such a very wide electrode will result in a small impedance signal when compared with that of a cell that is positioned over the edge of an electrode, where the field strength can be significantly higher. Preferably, an electrode element's width is between about 0.5 times and about 10 times the size (e.g., the width of an average cell when it spreads and attaches to the substrate) of cells used in an assay that uses the device. Preferably, for an electrode element that is part of an IDES or CCES, where the device is to be used for monitoring eukaryotic cells, such as mammalian cells, such as cancer cells, endothelial or epithelial cells, an electrode or electrode element is less than about 500 microns wide, and is preferably less than about 250 microns wide. In some preferred embodiments of the present invention, an electrode element is between about 20 microns and about 250 microns wide."

Thus, extensive experimentation was performed to determine electrode element width and the width to gap ratios that could be used in the electrode system of the instant invention for monitoring the electrode resistance or impedance. Figure **(42B)** shows exemplary data where paragraph **[00107]** concludes that

> "As indicated by the data, a significant increase in the cell number index is achieved with width/gap ratio of about 1.5 or higher."

Thus, based on the above development, consideration as for the electrode gap to cell size and as for the electrode width to cell size and based on the experimental data illustrated in Figure **(42B)**, Paragraph [00175] provides examples of ranges that were appropriate for the present system,

> "In the present application, it is preferred that the electrode gap between electrode elements should be designed with respect to the electrode width. ..... Preferably, the electrode element width is between 1.5 and 15 times the gap width. More preferably, the electrode element width is between 2 and 6 times the gap width; for example, if the electrode width is 90 microns at the widest point of each electrode, the gap width would be about 20 microns at the widest point of the gap between adjacent electrodes. For the present application, the electrode width can range from less than 5 microns to more than 10 mm. Preferably, the electrode width

> is in the range between 10 micron and 1 mm. More preferably, the electrode width is in the range between 20 micron and 500 micron."

Whilst the data in Figure **42(B)** showed that the "cell number index" signal would be significantly larger for the electrode width-to-gap ratio being 1.5 or greater, one should not have a very wide electrode width either. That was discussed in Paragraph **[00174]**, cited here as,

> "The width is also limited by the consideration that if an electrode element is very wide, a cell that is positioned over a central part of such a very wide electrode will result in a small impedance signal when compared with that of a cell that is positioned over the edge of an electrode, where the field strength can be significantly higher."

Thus, the proposed ratio between electrode width and electrode gap is for the aim of achieving large impedance signal upon cell attachment, making sure that the individual cells attached to the electrode would result in somewhat similar impedance signals. The Applicant's overall design goal is described in paragraph **[00164]**, cited as

> "It is an object of the present invention to reliably, sensitively, and quantitatively measure and monitor target molecules or cells which are in or suspected to be in sample solutions..." ."

There were additional technical difficulties beyond manipulation and design of the electrode array itself, but also in electrically connecting the array. Examples are discussed in paragraph [00176], which recites,

> "Thus, such connection traces may have an electrically insulating coating so that molecular reactions on or cell attachment to these connection trace regions will not result in a change in impedance between or among electrodes. In some embodiments, the electrode buses or electrically-conducting connection traces (e.g., 125 and 225 in Figure **1A** and **1B**) to connect the electrode elements may be located outside the bottom surface of a fluidic container or well that comprise the electrode structure. In this way, when sample solutions are added into the fluidic container or well, molecular reactions (or cell attachment) will not occur on such electrical connection traces. Taking the electrode structure 110 in **Figure 1A** as an example, the inner diameter of the arc-shaped, electrically conducting connection traces may have a diameter of 1.2 mm. This exemplary device is assembled to a plastic, cylinder shaped, fluidic container which has openings on both ends. The inner diameter of the cylinder-shaped fluidic container may be 1 mm. Using a double-sided adhesive (for example, a

pressure-sensitive-adhesive), the electrode device can be bond to the fluidic container. The electrode area is concentrically aligned with and bond to a circular end of the fluidic container. Thus, the 1.2 mm diameter will be located outside of the bottom surface of the container."

Another example is discussed in paragraph [00204], which recites,

"Electronic connection from such multi-well plates to external impedance analyzer present a significant challenge because of limited space on the bottom side of these plates. The electrode structures are facing upwards in operation. In one exemplary embodiment for connecting electrode structures to external impedance analyzers, the electronic connection pads are located at the ends of the electrode-containing substrates (see, for example, **Figure 12A** and **12B**). Because of very limited spaces available along the bottom edges of the multi-well plate, connectors used in the electronics industry cannot be directly used to such devices. In addition, because of the frame of the multi-well plate, there may not be space available for electronic connections from the top side to the connection pads at the ends of the electrode-containing substrates. For this reason, specific design is required for connecting the up-facing the connection pads to become bottom-facing. In one approach, a small PCB board (see **Figure 13A**) with straight-line conductor lines is down-facing and one end of all the conductor lines is conductively-bonded to the connection pads (see Figure **13B** and **13C**). Then the other end of the conductor lines can be accessed from the bottom. container."

Thus, the development of Applicants' invention required more than routine experimentation in view of one skilled in the art. Applicants were required to traverse many technical hurdles that did not have obvious answers. Neither Gerwen or Wolf included such extensive consideration of proper gap to electrode width ratios. This was in part because both Wolf and Gerwen operate differently than Applicants' invention.

E. Neither Wolf or Gerwen Provide a Device that Detects Impedance Changes Resulting From Attachment of the Cells to the Electrode Surface

Claim 1 provides a device for detecting cells on an electrode surface through measurement of impedance changes resulting from attachment of the cells to the electrode surface. Neither Wolf or Gerwen disclose such features.

Wolf has been discussed in previous Office Actions. Applicants incorporate by reference the description of Wolf and its deficiencies as set forth in the RCE, filed prior

to the present Office Action. To summarize, Wolf includes a plurality of sensors including at least one reference sensor, at least one electrical sensor and a macroporous interlayer. The macroporous interlayer is disclosed in column 2, line 63 to column 3, line 10,

> "The interlayer provided is in particular a macromolecular porous layer which on the one hand induces adhesion of the cells and on the other hand is proportioned in the pore size so as to be permeable for certain ions, molecules or cell areas. By way of example, an SiO_2 layer sputtered or applied to the measurement structure, an Al_2O_3 layer or a Ta_2O_5 layer can also be provided as the interlayer. By means of the structured interlayer, the electronic measurement structure is conditioned in such a way that the target cells or receptor cells accept the measurement structure as neighbor and become better attached to it. The porosity of the interlayer allows that the ions, molecules or cell areas to be measured of the target cells or receptor cells can reach the electrically active areas of the measurement structure."

Thus in Wolf, the cells do not directly contact the electrode surface but instead contact the macroporous interlayer, which is a porous layer sputtered over the measurement structure. Had Applicants' design been altered to include such features used in Wolf, the "structured, porous interlayer" would significantly affect the electrical field distribution generated by electrodes structures and affect the impedance measurements.

In addition, as discussed above, Gerwen discusses the measurement of molecular binding interactions and not the detection of cells. Thus the electrodes in Gerwen do not contact cells.

In addition, Claim 8 and 9 provide important embodiments having the electrode element widths between 0.5 times and 10 times cell size, and between 20 micron and 500 micron, respectively.

Applicants respectfully request the rejections be withdrawn and the claims allowed.

II.

Claims 4, 16-24, 29-32, 34, 44, 47-50, 289 and 298-308 are not obvious over Wolf (US 6280586) in view of Gerwen (US 6440662) and further in view of Wolf (US 6376233)

The Examiner has rejected claims 4, 16-24, 29-32, 34, 44, 47-50, and 298 as allegedly being obvious over Wolf (US 6280586) in view of Gerwen (US 6440662) as applied to claim 13, and further in view of Wolf (US 6376233).

Claim 4 provide important embodiment further comprising a plurality of receptacles, each of which is disposed on the substrate.

Claim 22 provide important embodiments having the electrode element widths between 0.5 times and 10 times cell size.

Claim 300 provides important embodiment of deriving a cell number index from the monitored impedance. Wolf and Gerwen appear to directly measure and compare impedance values. In contrast claims 300 and 301 include deriving a cell index. The cell index correlates to the amount and viability of the cells within the particular experiment. Thus the cell index provides more information than mere shifts in impedance values. A discussion of the cell index and its derivation is provided in paragraph [00286] and those that follow, which provides in part,

> "Based on the dependent relationship between the measured impedance, cell number (more accurately, the viable cell number, or attached cell number) and cell attachment status, it is possible to derive a so-called "cell number index" (or cell index) from the measured impedance frequency spectra."

The benefit of using Applicants' cell index is discussed throughout the application, including paragraphs [00296]-[00299], which provide,

> "There are several advantages of using "cell number index" to monitor cell growth and/or attachment and/or viability conditions.
>
> First, one can compare the performance of different electrode geometries by utilizing such cell number index.
>
> Secondly, for a given electrode geometry, it is possible to construct "calibration curve" for depicting the relationship between the cell number and the cell number index by performing impedance measurements for different number of cells added to the electrodes (in such an experiment, it is important to make

sure that the seeded cells have well-attached to the electrode surfaces). With such a calibration curve, when a new impedance measurement is performed, it is then possible to estimate cell number from the newly-measured cell number index.

Thirdly, cell number index can also be used to compare different surface conditions. For the same electrode geometry and same number of cells, a surface treatment given a larger cell number index indicates a better attachment for the cells to the electrode surface and/or better surface for cell attachment."

With respect to claim 301, the cell index can be derived from at least one of four processes provided. Neither Wolf or Gerwen demonstrate a cell index or these specific processes for derivation.

Applicants have amended claim 1, from which claims 4, 16-24, 29-32, 34, 44, 47-50, 289 and 298-308 depend. Wolf 6376233 does not cure the deficiencies in a proper obviousness rejection over Wolf 6280586 in view of Gerwen. Thus claims 4, 16-24, 29-32, 34, 44, 47-50, 289 and 298-308 are also not obvious over Wolf in view of Gerwen and further in view of Wolf.

Thus, Applicants respectfully request the rejection be withdrawn and the claims allowed.

III.

Claim 35 is Not Obvious Over Wolf (US 6280586) in view of Gerwen (US 6440662) as applied to claim 1, and further in view of Surridge (US20030116447)

The Examiner has rejected claim 35 as allegedly being obvious over Wolf in view of Gerwen and further in view of Surridge (US20030116447).

Applicants have amended claim 1, from which claim 35 depends. Surridge does not cure the deficiencies in a proper obviousness rejection over Wolf in view of Gerwen. Thus claim 35 is not obvious over Wolf in view of Gerwen and further in view of Surridge.

Thus, Applicants respectfully request the rejection be withdrawn and the claim 35 allowed.

IV.

Claim 37 is Not Obvious Over Wolf (US 6280586) in view of Gerwen (US 6440662) as applied to claim 1, and further in view of Gomez (US 20030157587)

The Examiner has rejected claim 37 as allegedly being obvious over Wolf in view of Gerwen and further in view of Gomez

Applicants have amended claim 1, from which claim 37 depends. Gomez does not cure the deficiencies in a proper obviousness rejection over Wolf in view of Gerwen with respect to claim 1. Thus claim 37 is not obvious over Wolf in view of Gerwen and further in view of Gomez.

Thus, Applicants respectfully request the rejection be withdrawn and the claim 37 allowed.

V.

Claims 41 and 42 are not obvious over Wolf (US 6280586) in view of Caillat (US 6630359) as applied to claim 40, and further in view of Sugihara (US 6132683)

The Examiner has rejected claims 41 and 42 as allegedly being obvious over Wolf in view of Gerwen and further in view of Sugihara.

Applicants have amended claim 1, from which claims 41 and 42 depend. Sugihara does not cure the deficiencies in a proper obviousness rejection over Wolf in view of Gerwen with respect to claim 1. Thus claim 41 and 42 is not obvious over Wolf in view of Gerwen and further in view of Sugihara.

Thus, Applicants respectfully request the rejection be withdrawn and the claims 41 and 42 allowed.

Response to Double Patenting Rejections

VI.

The Examiner has issued a provisional obviousness-type double patenting rejection of claims 1, 4, 25, 38-40 and 72 over copending Application No. 11/055,639 in view of Gerwen (US 6440662).

Applicants have amended claim 1 to recite the electrode gap is at least 3 microns wide. Applicants have amended claim 72 to recite each of the gaps is at least 3 microns wide.

Application no. 11/055,639 in view of Gerwen does not recite the combination of all of these elements and thus Applicants respectfully request the provisional double patenting rejection be withdrawn.

VII.

The Examiner has issued a provisional obviousness-type double patenting rejection of claims 1, 4, 25, 38-40 and 72 over copending Application No. 10/987,732 in view of Gerwen (US 644062)

Applicants have amended claim Applicants have amended claim 1 to recite the electrode gap is at least 3 microns wide. Applicants have amended claim 72 to recite each of the gaps is at least 3 microns wide.

Application no. 10/987,732 in view of Gerwen does not recite the combination of all of these elements and thus Applicants respectfully requests the provisional double patenting rejection be withdrawn.

Conclusion

In view of the amendments and argument set forth above, Applicants respectfully request all rejections be withdrawn and a notice of allowance be issued in this case.

Respectfully submitted,

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